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TITLE: Cell of Origin and Cancer Stem Cell Phenotype in Medulloblastomas

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14. ABSTRACT The goal of this project is to test our hypothesis that cellular context in which tumors initiate may have a dominant role over some oncogene function in determining molecular phenotypes. To test this hypothesis, we proposed to transform neural stem cells (NSCs) and neural progenitor cells (NPCs) by expressing an activated form of *Notch1* (N1ICD) or oncogenic *PIK3CA* (PIK3CA*) in the developing mouse cerebellum, using cell type- specific Cre drivers *(En2-Cre for NSCs and Atoh1-creER for NPCs)*. During this funding period, we were successful in intercrossing *N1ICD, En2-Cre*, *Atoh1-cre*, and *p53* strains to generate *N1ICD;En2-cre;p53-/-* and *N1ICD;Atoh1-CreER;p53-/-* mice. We are currently aging these mice to collect medulloblastomas for molecular analyses. For PIK3CA*-induced models, we first analyzed the effect of PIK3CA* expression in different cellular compartments in the developing brain since this is a new model and the effects of oncogenic PIK3CA* expression in the developing brain is unknown. Our analyses showed that our PIK3CA* transgenic model is functional and that oncogenic PIK3CA* expression in the developing brain affects proliferation and differentiation. We are intercrossing PIK3CA* mice with *Atoh1-cre, En2-cre, and p53-/-* mice to generate PIK3CA**;Atoh1-CreER;p53-/-* and *PIK3CA**;Atoh1-CreER;p53-/-* mice to generate spontaneous medulloblastomas.

15. SUBJECT TERMS cancer stem cells, medulloblastoma, targeted therapy, therapy resistance, pediatric cancer, brain tumor, Notch1, PIK3CA, cell of origin, molecular subtypes, neural stem cells, neural progenitor cells, tumor initiation.

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1. INTRODUCTION:

The goal of this project is to test our **hypothesis that cells in various stages of maturation in the developing brain produce tumors with distinct biological characteristics when transformed by the same oncogenic event**. Validation of this hypothesis would have significant clinical implications, as it could lead to identification of specific biological characteristics that could serve as novel and effective therapeutic targets. Our hypothesis is based on our recent study that showed that cancer stem cells (CSCs) arising from neural stem cells, the most primitive cells in the developing brain, are resistant to targeted therapies, while cancer stem cells derived from more mature progenies of neural stem cells are sensitive to the same drugs. In other words, responsiveness of cancer stem cells to targeted therapies varied greatly depending on the cell type in which tumor initiation occurred. If this novel discovery is generalizable, it would suggest that we will need to analyze cancer stem cells (rare cells in the tumor) and not just the bulk tumor cells (current practice) to identify therapy combinations that will eradicate both cancer stem cells and non-stem (bulk) tumor cells. To test the general applicability of our findings, we will use two new models of medulloblastoma (induced by expression of mutant oncogenes) to validate the role of cell-of-origin in determining the cancer stem cell phenotype. Results of this project will transform the way we approach therapy design and therapy resistance as well as methods used to diagnose patients.

2. KEYWORDS:

cancer stem cells, medulloblastoma, targeted therapy, therapy resistance, pediatric cancer, brain tumor, Notch1, PIK3CA, cell of origin, molecular subtypes, neural stem cells, neural progenitor cells, tumor initiation.

3. ACCOMPLISHMENTS:

Major goals of the project:

The stated goals of this project are to: 1) test the general applicability of our observation across multiple tumor models in which different oncogenic hits initiate tumor formation and 2) test our hypothesis that cells in different stages of maturation in developing organs produces tumors with distinct molecular and cellular characteristics even when the initiating oncogenic event is the same.

To test the general applicability of our novel hypothesis, we will transform neural stem cells (NSCs) and neural progenitor cells (NPCs) in the developing mouse cerebellum using cell stage- specific Cre drivers (*En2-Cre or GFAP-cre for NSCs* and *Atoh1-creER or Olig2-cre for NPCs*). We will expressed activated *Notch1* (N1ICD) or an oncogenic mutant form of PIK3CA (PIK3CA*) in *p53*-/- brains. We will analyze both bulk tumors and CSCs from each of these models and compare their molecular and cellular characteristics, including CSC culture behavior and AKT activation. We will also compare molecular profiles of bulk tumors and CSCs of these tumors to those from other murine models we have analyzed previously to determine whether the oncogene or the cellular context plays a more dominant role in driving the molecular phenotypes by unsupervised clustering analyses.

What was accomplished:

During this period, we focused on generating new models of medulloblastoma by activating N1ICD and PIK3CA* in cerebellar NSCs and NPCs in the developing mouse brain.

We previously published that activated Notch1 (N1ICD) expression in the developing brain induces apoptosis due to DNA damage and p53 activation. When p53 is genetically deleted, ~40% of *N1ICD;GFAP-cre;p53-/-* mice developed spontaneous medulloblastomas (Natarajan et al., 2013). To generate medulloblastomas that arise from transformed NSCs, we intercrossed *N1ICD, En2-Cre*, and *p53* strains to generate *N1ICD;En2-cre;p53-/-* mice. To activate the same transgene in NPCs in the external granule layer (EGL), we intercrossed *N1ICD, Atoh1-CreER,* and *p53* strains to generate *N1ICD;Atoh1-CreER;p53-/-*. We are currently aging these mice to collect medulloblastoma samples for analysis.

Because the reviewers had asked for (and DoD approved) switching out *Xrcc2-/-*induced medulloblastoma model (proposed in the original submission) with PIK3CA*-induced medulloblastoma model, we are behind schedule in terms of generating tumors. We had to first carefully analyze the effects of PIK3CA* expression in different cellular compartments in the developing brain. As shown in Figure 1, expression of mutant PIK3CA* in the developing embryo brain (by Nestin-Cre) induced severe dysplasia (Fig 1A, B), and *PIK3CA;Nestin-cre* mice died with hydrocephalus by weaning age. We validated elevated PIK3CA signaling in these brains by increased pAKT and pS6 expression in transgenic brains (Fig 1C, D). *PIK3CA** expression in slightly more mature neuroepithelium (by GFAP-Cre) induced milder dysplasia with prominent rosette formation in the neuroepithelium (Fig 1E), but still resulted in hydrocephalus and lethality by weaning age. Interestingly, PIK3CA* expression in committed neural progenitors (by Ngn1-cre) did not result in dysplasia although the transgenic brains are megacephalic, Fig 1F, G). These mice also died around 2 months of age of unknown reasons. These analyses showed that the PIK3CA* transgenic model we use is functional and that oncogenic PIK3CA expression in the developing brain affects proliferation and differentiation, as anticipated.

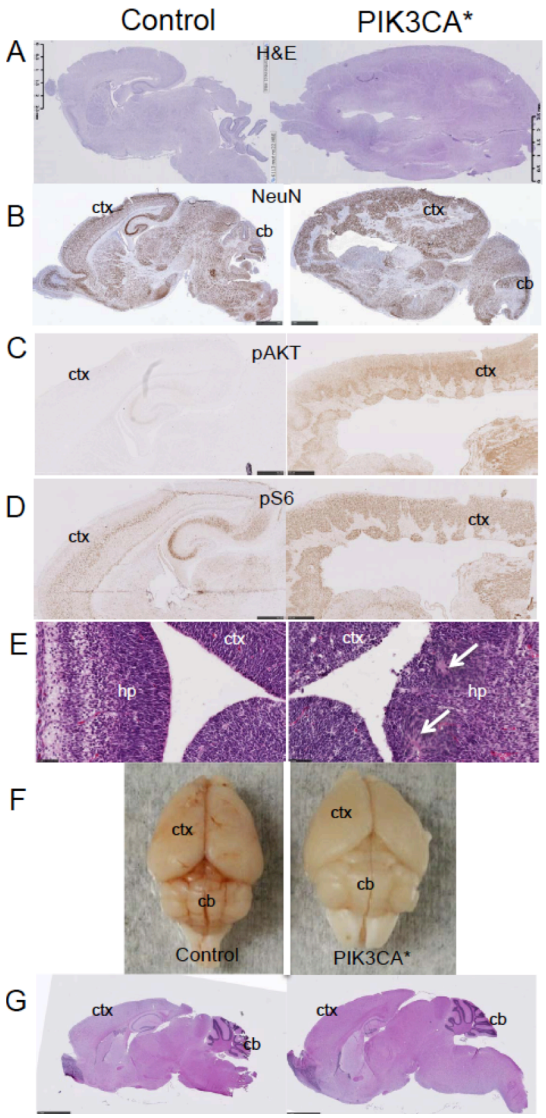

Figure 1. Postnatal day 5 PIK3CA*;Nestin-cre and control brains stained with (A) H&E, (B) NeuN, a neuronal marker, (C) pAKT, and (D) pS6. (E) E15.5 PIK3CA*;GFAP-Cre brain stained with H&E showing rosettes in neuroepithelium. Gross (F) and H&E (G) stained images of control (left) and PIK3CA*;Ngn1-cre brain (right) at 2 months showing megacephaly. Abb: ctx=cortex, cb= cerebellum, hp= hippocampus

To generate new medulloblastoma models induced by PIK3CA* expression, we directed PIK3CA* expression in the developing cerebellum by mating PIK3CA* mice to the En2-Cre driver. En2-Cre is active in mid/hind brain neuroepithelium from very early on (E9.0 onwards). *PIK3CA*;En2-cre* mice are viable (>240 days) but they have hypoplastic vermis and hyperplastic superior collilus (Fig 2A), suggesting that the effects of PIK3CA* expression is cell context-specific. Furthermore, cerebellar hemispheres were disorganized (Fig 2B), and marker analyses for activated PI3K pathway (pS6, Fig 2C), purkinje neurons (calbindin, Fig 2D), and proliferation (Ki67, Fig 2E) suggest that aberrant elevation of PIK3CA signaling affects cell proliferation/survival, differentiation and migration. Note that a change in proliferation is not apparent at this age. Together, these results indicate that PIK3CA* expression in early cerebellar stem cells may result in oncogene-induced apoptosis or senescence at an early age. We are currently analyzing PIK3CA* expression in cerebellar NPCs, using *Atoh1-CreER* inducible driver in EGL progenitor cells. We will determine whether embryonic and postnatal day EGL progenitor cells respond similarly as NCSs to PIK3CA* expression and whether deleting the p53 tumor suppressor gene function will result in spontaneous medulloblastoma formation (in both *En2-Cre* and *Atoh1-CreER* models).

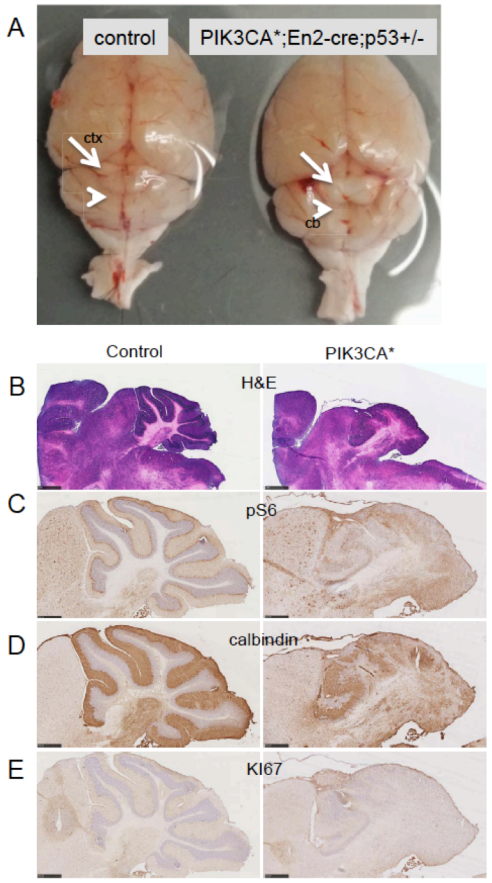

Figure 2. Littermate control and PIK3CA*'En2-cre;p53+/- brains at 6 months (A) gross images, (B) H&E, (C) pS6, (D) calbindin, and (E) KI67 staining. Arrows in A point to inferior colliculus, arrowheads point to vermis.

Training opportunities: N/A

Results dissemination: Nothing to report

Plan for the next reporting period:
We will continue to intercross to generate triple transgenic mice and age them to collect at least 10 tumors of each genotype (*N1ICD;Atoh1-CreER;p53, N1ICD;En2-Cre;p53, PIK3CA*;Atoh1-CreER;p53,* and *PIK3CA*;En2-Cre;p53*). We will analyze their transcriptomes to determine whether the cell of origin or the oncogenic function plays a dominant role in determining the molecular phenotypes of medulloblastomas.

4. IMPACT:
Impact of the principal and other disciplines: Nothing to report

Impact on technology transfer: Nothing to report

Impact on Society: Nothing to report

5. CHANGES/PROBLEMS:
Problems or delays:

This project is a little delayed due to two main reasons. One, we observed higher than anticipated incidence of sarcoma formation from mice in p53+/- or p53-/- backgrounds. We had to sacrifice triple transgenic mice before they could form brain tumors; hence, we are behind schedule in terms of collecting spontaneous medulloblastomas. To bypass this limitation, we started crossing floxed-p53 mice to N1ICD and PIK3CA* mice so that we can delete p53 only in cells that are also expressing N1ICD or PPIK3CA* oncogenes in the brain. The second reason for the delay is that the reviewers had asked us to change the second oncogenic event (*Xrcc2* deletion) to a more clinically–relevant genetic event (we chose PIK3CA mutation). This change was approved pre-award by DoD. However, since this is a new model, we had to do more model characterization than anticipated, which caused some delay.

Changes with significant impact on expenditure: Nothing to report

Changes to human subjects, animals, or agents: Nothing to report

6. PRODUCTS: Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name	Kyuson Yun
Project Role	Principal Investigator
Researcher Identifier (NIH Commons ID)	KYUSONYUN
Nearest person month worked	3
Contribution to Project	overall supervision, experimental design and analysis.
Funding Support	N/A

Name	Kin-Hoe Chow
Project Role	Postdoctoral Associate
Researcher Identifier (NIH Commons ID)	KINHOECHOW
Nearest person month worked	11
Contribution to Project	generation and analysis of new medulloblastoma models
Funding Support	N/A

Name	Keiko Yamamoto
Project Role	Research Assistant
Researcher Identifier	N/A
Contribution to Project	animal husbandry to generate new models of medulloblastoma
Funding Support	N/A

Name	Rachael McMinimy
Project Role	Co-Op Associate
Researcher Identifier	N/A
Nearest person month worked	1
Contribution to Project	histological analysis of new medulloblastoma models
Funding Support	N/A

Has there been a change in the active other support of the PD/PIs or senior/key personnel since the last reporting period?

Yes. Dr. Yun's current other support is detailed below (changes are indicated in italics.)

Active

Supporting Agency:	Oliver S. and Jennie R. Donaldson Charitable Trust DONALDSON-FY13-KY-01	**PI:**	Yun
Project Title:	Cancer Risk Factors and Cell Type: Elucidating Brain Cancer Formation		
Role:	Principal Investigator	**Effort:**	1.20 CM
Entire Project:	12/17/2013 - 12/16/2015		
Current Year:	12/17/2014 - 12/16/2015		
Project Goals:	The goal of this project is elucidate the underlying mechanism of differential vulnerability of neural stem and neural progenitor cells to oncogenic insult and cellular transformation.		
Specific Aims:	1. Test the hypothesis that cellular context is dominant over some oncogene function in vivo.		
Overlap:	None		
Contracting/ Grants Officer:	Allen Mast, Corporate Trustee		

Supporting Agency:	*NIH/NCI 1 R21 CA191848-01A1*	***PI:***	*Chuang*
Project Title:	*Dissection of Tumor Evolution Using Patient-Derived Xenografts*		
Role:	*Co-Investigator*	***Effort:***	*0.60 CM*
Entire Project:	*07/01/2015 - 06/30/2017*		
Current Year:	*07/01/2015 - 06/30/2016*		
Project Goals:	*The goal of this exploratory study is to test and apply patient-derived xenografts (PDX) as an improved system to quantify rates of tumor subclonal population evolution.*		
Specific Aims:	*1: Spatial and Temporal Dissection of Subclonal Heterogeneity in Breast Cancer Xenografts - a. Characterization of spatially and temporally separated breast cancer xenografts; b. Computational identification and analysis of subpopulations; c. Validation of subpopulations by single-cell sequencing; 2: Determination of Subclonal Evolution During Drug Treatment - a. Genomic and histological characterization of spatially and temporally separated xenografts under drug treatment; b. Identification, analysis, and validation of*		

	subpopulations relevant to therapy response.
Overlap:	*None*
Contracting/ Grants Officer:	*Sarah M. Lee - sarah.lee@nih.gov*

Supporting Agency:	*Maine Technology Institute SG5424*	***PI:***	*Yun*
Project Title:	*Development of Novel Anti-cancer Agents*		
Role:	*Principal Investigator*	***Effort:***	*0.12 CM*
Entire Project:	*07/01/2015 - 06/30/2016*		
Current Year:	*07/01/2015 - 06/30/2016*		
Project Goals:	*The main goal of this study is to develop new Yap1 inhibitors (derivatives of VP) with significantly enhanced solubility, and consequently increased cellular uptake, and dark activity.*		
Specific Aims:	*1. develop new Yap1 inhibitors (derivatives of VP) with significantly enhanced solubility, and consequently increased cellular uptake, and dark activity.*		
Overlap:	*None*		
Contracting/ Grants Officer:	*Shane Beckim - sbeckim@mainetechnology.org*		

Completed

Supporting Agency:	*American Brain Tumor Association*	***PI:***	*Yun, Kyuson*
Project Title:	*Predicting Therapy Resistance Based on Cancer Stem Cell Phenotypes*		
Role:	*Principal Investigator*	***Effort:***	*0.60 CM*
Entire Project:	*07/01/2013 - 06/30/2014*		
Current Year:	*07/01/2013 - 06/30/2014*		

Supporting Agency:	*Maine Cancer Foundation*	***PI:***	*Yun, Kyuson*
Project Title:	*Development of Ex Vivo Organotypic Slice Culture Systems for Cancer Studies*		
Role:	*Principal Investigator*	***Effort:***	*1.20 CM*
Entire Project:	*07/01/2013 - 06/30/2015*		
Current Year:	*07/01/2013 - 06/30/2014*		

Supporting Agency:	*The Jackson Laboratory Director's Innovation Fund*	***PI:***	*Yun, Kyuson*
Project Title:	*Postdoctoral Associate Support*		
Role:	*Principal Investigator*	***Effort:***	*0.12 CM*
Entire Project:	*08/01/2013 - 07/31/2014*		
Current Year:	*08/01/2013 - 07/31/2014*		

Supporting Agency:	*American Cancer Society* *118571-RSG-10-042-01-DDC*	***PI:***	*Yun, Kyuson*
Project Title:	*S100a4 Expression and Function in Brain Cancer Stem Cells*		
Role:	*Principal Investigator*	***Effort:***	*2.40 CM*
Entire Project:	*01/01/2010 - 12/31/2014*		
Current Year:	*01/01/2013 - 12/31/2014*		

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS: none

9. APPENDICES: N/A